

Liquid Chromatography

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Introduction

In general, inorganic ions cannot be detected using absorbance spectroscopy or electrochemistry. As a result, determinations of inorganic ions by liquid chromatography (LC) possess many characteristics distinct from determinations of organic compounds by LC. These differences are most noticeable in the detection methodologies used, but also feed back into the column design and operation. This article focuses on the distinct aspects of LC determinations of inorganic ions, and provides an introduction to approved methodologies for inorganic ion analysis. For further reading, the monograph by Haddad and Jackson and the recent special issue of *Journal of Chromatography A* (volume 789) devoted to chromatography of inorganic ions are strongly recommended.

Instrumentation for Inorganic Ion Analysis

General

Figure 1 shows a schematic diagram of a high performance liquid chromatograph (HPLC) for inorganic ion analysis. This instrument is commonly referred to as an ion chromatograph. The most distinguishing difference from a typical HPLC is the post-column reactor. Nonetheless, there are a number of other subtle differences. The pump, injector and tubing are essentially standard HPLC components. The only significant difference is that for inorganic ion analysis these components are generally constructed of metal-free materials, such as poly-

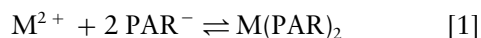
etheretherketone (PEEK). PEEK is extremely chemically inert, flexible, inexpensive and capable of withstanding high pressures.

Column

Separations of inorganic ions are performed using ion exchange. However, classical gel type (polystyrene-divinylbenzene) ion exchangers are not used. Such resins possess too high a capacity and the mass transfer is too slow to allow the low eluent strengths and high separation efficiencies required. Rather, columns for inorganic ion analysis are generally of two types. Firstly, and most commonly, the columns are packed with pellicular particles. Pellicular particles have a solid core with ion exchange sites only on the outer surface of the particle. Alternatively, dynamic ion exchange columns can be prepared from reversed-phase packings (C_{18}) by addition of an ion pair reagent to the mobile phase. In either case the ion exchange capacity of the columns is low, typically 20–200 μeq per column.

Post-column Reactor

As stated above, the most distinguishing feature of an HPLC for inorganic ion analysis is the post-column reactor. Its purpose is to facilitate detection of the inorganic ions. The simplest post-column reaction is the formation of a coloured product which can be measured with a spectrophotometer. For example, transition and lanthanide metal ions (M) can be monitored using post-column reaction with 4-(2-pyridylazo)resorcinol (PAR):



A reagent stream of excess PAR is added to the effluent from the ion exchange column. The

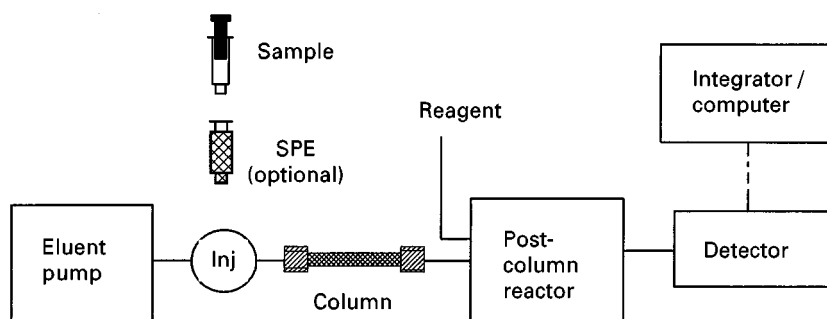
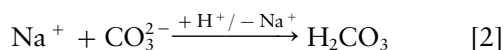


Figure 1 Schematic diagram of a high performance liquid chromatograph for analysis of inorganic ions. SPE, solid-phase extraction.

post-column reaction should ideally be instantaneous, as with PAR, allowing use of a simple T-connector as the post-column reactor. Once the colourless metal ions present are complexed by the PAR, the PAR absorbance undergoes a bathochromic shift. That is, in the absence of metal ions, the PAR reagent absorbs little light at ~ 510 nm, whereas metal-PAR complexes absorb intensely at this wavelength. This absorbance can be directly related to the metal ion concentration.

A more complex post-column reaction is suppression, which is used in conjunction with conductivity detection. All inorganic ions conduct electricity. Thus, conductivity is a universal detector for these species. However, the eluents used in ion exchange chromatography are also conducting. The function of the post-column suppressor is to eliminate (suppress) the background conductivity of the eluent, or at least reduce it to an insignificant level. Typical suppressors are membrane-based, although fibre- and column-based suppressors were used in older systems. The suppression process can be illustrated using the example of the determination of inorganic anions in a sodium carbonate eluent, as is used in Environmental Protection Agency (EPA) Method 300.0 and American Society for Testing and Materials (ASTM) Method D 4327. The membrane in a suppressor for anion chromatography is a cation exchange membrane. Eluent from the column flows on one side of the membrane, while a regenerant flows on the other side. The regenerant is typically H_2SO_4 . H^+ from the regenerant and Na^+ from the eluent freely exchange through the cation exchange membrane. Anions cannot pass through the membrane due to charge repulsion. As a result of exchange of H^+ for Na^+ in the eluent stream, the carbonate eluent is protonated, minimizing the background conductivity.



Carbonic acid is however a weak acid and does undergo some dissociation back to H^+ and HCO_3^- . However, this dissociation only results in a small residual ($\sim 15 \mu\text{S}$) background conductivity. Samples do not possess this background conductivity. Thus, a 'water dip' is observed at the dead volume of the column (0.7 min in Figure 2) when the water from the sample elutes from the column. The weak acid character of carbonic acid can also cause some nonlinearity in calibration curves for inorganic anions when suppressed conductivity detection is used.

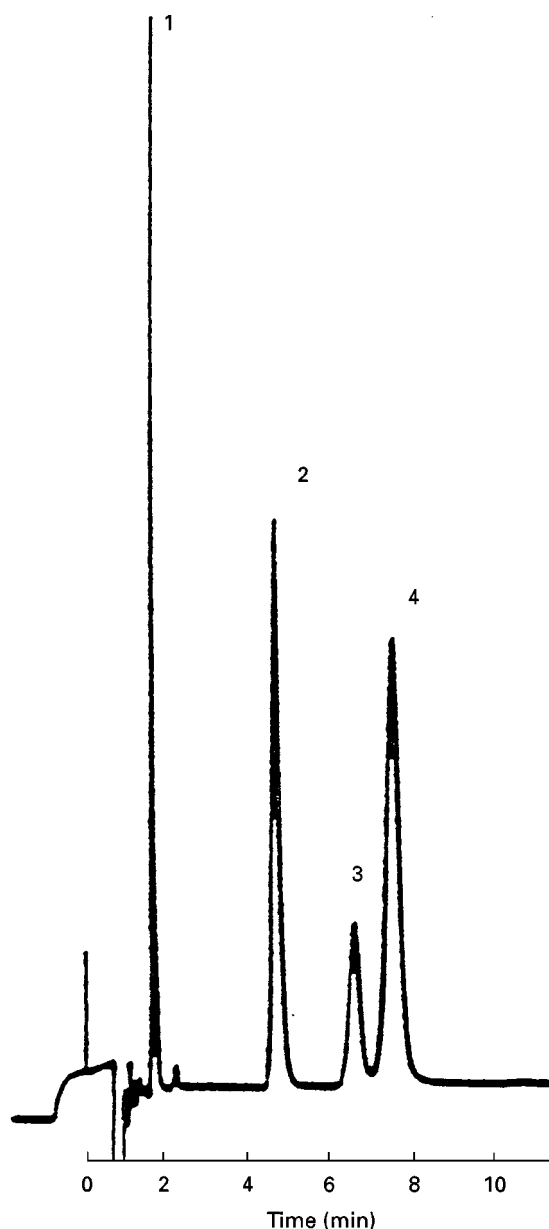


Figure 2 Separation of standard inorganic anions using pre-concentration as per ASTM method D 5542. Peaks: 1, $5 \mu\text{g L}^{-1}$ chloride; 2, $20 \mu\text{g L}^{-1}$ nitrate; 3, $20 \mu\text{g L}^{-1}$ phosphate; 4, $20 \mu\text{g L}^{-1}$ sulfate. Experimental conditions: column, Dionex AS4A; eluent, $0.75 \text{ mmol L}^{-1} \text{ NaHCO}_3/2.2 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3$; flow rate, 2.0 mL min^{-1} ; detection, suppressed conductivity; injection volume, 10 mL on to a TAC-1 concentrator column. (Courtesy of ASTM.)

Special Considerations for Inorganic Ion Analysis

Water

Water used in the preparation of standards and eluents for routine determinations should be freshly distilled and deionized, and meet the specifications

for ASTM Type II water ($1 \text{ M}\Omega \cdot \text{cm}$). However, $1 \text{ M}\Omega \cdot \text{cm}$ water may contain up to 200 p.p.b. of any anion. Therefore, for trace anion analyses, ASTM Type I water ($18 \text{ M}\Omega \cdot \text{cm}$) is recommended. Modern deionization systems using nuclear-grade ion exchange resins to polish out inorganic impurities provide water of this grade which contains down to 20 p.p.t. chloride, 100 p.p.t. sodium and 50–60 p.p.t. ammonium.

Eluents

All eluents should be filtered through $0.2 \mu\text{m}$ membranes before use. Many ion chromatographic eluents are excellent growth media for algae. Therefore eluents should be stored at 4°C and not kept for longer than 1 month.

Sample Handling

Many samples possess acidic, alkaline or saline matrices which can disrupt liquid chromatographic determinations of inorganic ions. In such cases it is essential that the matrix components be removed prior to analysis. Solid-phase extraction (SPE) is a convenient means of eliminating such interferents. A SPE device is generally a small column or filter containing a chromatographic medium which fits on

to a sample syringe. Typical SPE cartridges for inorganic ion analysis contain cation exchange resin in the H^+ form to reduce sample pH, in the Ag^+ form to selectively precipitate halides and in the Ba^{2+} form to precipitate sulfate; anion exchange resin in the OH^- form to neutralize acidic samples; and hydrophobic materials such as polystyrene to remove hydrophobic matrix components. Passage of the sample through the SPE and directly into the injector provides a quick and convenient means of eliminating deleterious matrix components.

Methods for Inorganic Ion Analysis

A reliable source of methods for inorganic ion analysis is the approved regulatory methodologies. Table 1 provides a brief summary of approved methods from the US EPA and the ASTM. Other agencies, such as the National Institute for Occupational Safety and Health (US) and the Occupational Safety and Health Administration (US), also have approved methods for determination of air-borne species such as SO_2 , Br_2 , Cl_2 , NO_x and organoarsenics by conversion into aqueous inorganic ions and determination by ion chromatography.

Another rich source of information are vendor application notes. On-line listings of these application

Table 1 Regulatory methods for inorganic ion analysis by liquid chromatography

Method	Analytes ^a	Specified matrices
<i>EPA^b</i>		
218.6	Cr(VI)	Drinking water, groundwater, industrial wastewater effluents
300.0 A	F^- , Cl^- , NO_2^- , Br^- , NO_3^- , HPO_4^{2-} , SO_4^{2-}	Drinking water, surface water, wastewater, groundwater, reagent water, solids (after extraction), leachate
300.0 B	ClO_2^- , ClO_3^- , BrO_3^-	Drinking water and reagent water
300.6	Cl^- , NO_3^- , HPO_4^{2-} , SO_4^{2-}	Wet deposition (rain, snow, sleet, hail)
300.7	Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+}	Wet deposition (rain, snow, dew, sleet, hail)
A-1000	Cl^- , SO_4^{2-}	Drinking water
B-1012	NO_2^- , NO_3^-	Wastewater
<i>ASTM^c</i>		
D4327	F^- , Cl^- , NO_2^- , Br^- , NO_3^- , HPO_4^{2-} , SO_4^{2-}	Drinking water and wastewater
D4856	H_2SO_4	Workplace air samples
D5085	Cl^- , NO_3^- , SO_4^{2-}	Wet deposition (rain, snow, dew, sleet, hail)
D5257	Cr(VI)	Waste, drinking and surface waters
D5542 A	Cl^- , HPO_4^{2-} , SO_4^{2-}	High purity water
D5542 B	F^- , acetate, formate	High purity water
E1787	F^- , Cl^- , Br^- , ClO_3^- , NO_3^- , HPO_4^{2-} , SO_4^{2-}	Caustic soda (NaOH) and caustic potash (KOH)

^a Analytes listed in order of elution. Only analytes for which the method has been approved are listed. Other ions may be separated and detected using these methods.

^b EPA refers to the United States Environmental Protection Agency, Environmental Monitoring and Systems Laboratory, Cincinnati, OH 45268 USA. Copies of methods can be obtained from Superintendent of Documents, US Government Printing Office, Washington, DC 20402, USA.

^c STM refers to the American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103-1187 USA (telephone: (215) 299-5400; facsimile: (215) 977-9679). Methods are published in the *Annual Book of ASTM Standards*.

notes are available from a number of companies, such as Alltech Associates, Dionex Corporation, Metrohm and Waters Corporation.

Anions by Suppressed Conductivity

Many of the regulatory procedures listed in Table 1 for standard anions use suppressed conductivity ion chromatography and low capacity (20–35 μeq per column) ion exchange columns. Bicarbonate/carbonate eluents are used for the inorganic acid anions (EPA 300.0 and 300.6; ASTM D4327, D5085, D5542 A and E1787). Figure 2 shows the separation of standard anions using ASTM Method D 5542 with a bicarbonate/carbonate eluent on a Dionex AS4A column. Similar elution orders are observed with

other ion chromatographic columns. However, separation selectivities do vary with the column capacity, hydrophobicity and structure. Thus, the precise eluent concentrations and selectivities depend upon the specific column used.

Method B of the ASTM D 5542 protocol recommends the use of 5 mmol L^{-1} sodium tetraborate, for separation of fluoride, acetate and formate. These anions are very weakly retained, and so a weak eluent is required to separate them from each other and from the water dip.

Detection limits for these regulatory methods are listed in Table 2. Using a 50 μL injection loop, low parts per billion (p.p.b., $\mu\text{g L}^{-1}$) are achieved using suppressed conductivity. This equates to less than

Table 2 Performance of regulatory methods for inorganic ion analysis by liquid chromatography with conductivity detection

Ion	Suppressed conductivity		Direct conductivity detection limit (p.p.m.)
	Approved range (p.p.m.)	Detection limit (p.p.m.)	
Direct injection			
Fluoride	0.3–8 ^a	0.01 ^a	0.04 ^b
Chloride	0.8–26 ^a	0.02 ^a	0.02 ^b
Nitrite	0.4–12 ^a	0.004 ^a	0.04 ^b
Bromide	0.6–21 ^a	0.01 ^a	0.06 ^b
Nitrate	0.4–14 ^a	0.002 ^a	0.04 ^b
o-phosphate	0.7–23 ^a	0.003 ^a	0.15 ^b
Sulfate	2.9–95 ^a	0.02 ^a	0.10 ^b
Chlorite ^c	Not stated	0.01	
Bromate ^c	Not stated	0.02	
Chlorate ^c	Not stated	0.003	
Sodium ^d	0.03–1.00	0.03	
Ammonium (as NH ₄ ⁺) ^d	0.03–2.00	0.03	
Potassium ^d	0.01–1.00	0.01	
Magnesium ^d	0.02–1.00	0.02	
Calcium ^d	0.02–3.00	0.02	
Preconcentration ^e			
Fluoride	0–0.014	0.0007	
Acetate	0–0.4	0.007	
Formate	0–0.35	0.006	
Chloride	0–0.024	0.0008	
Phosphate	0–0.04	Not determined	
Sulfate	0–0.6	0.002	

^a From ASTM D5085 and EPA 300.0 A Column, Dionex AS4A; eluent, 1.7 mmol L^{-1} NaHCO_3 /1.8 mmol L^{-1} Na_2CO_3 ; flow rate, 2.0 mL min^{-1} ; detection, suppressed conductivity; injection volume, 50 μL .

^b From EPA A-1000. Column, Waters IC-Pak Anion; eluent, 1.5 mmol L^{-1} sodium gluconate/5.8 mmol L^{-1} boric acid/1.3 mmol L^{-1} sodium tetraborate/0.5% glycerine/2% butanol/6% acetonitrile (pH 8.5); flow rate, 1.2 mL min^{-1} ; detection, direct conductivity; injection volume, 100 μL .

^c From US EPA method 300.0 B. Column, Dionex AS9A; eluent, 1.7 mmol L^{-1} NaHCO_3 /1.8 mmol L^{-1} Na_2CO_3 ; flow rate, 1.0 mL min^{-1} ; detection, suppressed conductivity; injection volume, 50 μL .

^d From US EPA method 300.7. Column, Dionex CS1; eluent, 0.005 mol L^{-1} HCl for monovalent ions and 0.005 mol L^{-1} HCl/0.004 mol L^{-1} meta phenylenediamine dihydrochloride (MPDA-2HCl); flow rate, 2.3 mL min^{-1} ; detection, suppressed conductivity; injection volume, 100 μL .

^e From ASTM D5542. Column, Dionex AS4A; flow rate, 2.0 mL min^{-1} ; detection, suppressed conductivity; injection volume, 20 mL on to an AG-4A concentrator column. Eluent, 5 mmol L^{-1} sodium tetraborate for F^- , CH_3COO^- and HCOO^- , 0.75 mmol L^{-1} NaHCO_3 /2.2 mmol L^{-1} Na_2CO_3 for all other ions.

$1 \mu\text{mol L}^{-1}$ concentration in solution, or to less than 1 ng of anion injected. Much lower concentration detection limits can be achieved by preconcentrating the sample onto a small low capacity pre-column. In ASTM Method D5542 (Figure 2), 10 mL of high purity water is concentrated onto a pre-column. Such preconcentration can yield sub-p.p.b. detection limits. At such levels, proper handling of ultra pure water samples is essential to avoid contamination. Appropriate handling procedures are covered in ASTM Method D4453.

Anions by Direct Conductivity

Suppressed conductivity is the most common means of monitoring inorganic anions. However, approved methods also exist which do not use suppression. Rather these procedures measure the conductivity of the column effluent directly. EPA Method A-1000 has been approved for the determination of chloride and sulfate in drinking water. Figure 3A shows the separation achieved using a Waters IC-Pak anion column with a pH 8.5 borate/gluconate eluent.

Detection limits for direct conductivity detection are given in Table 2. In the analysis of drinking waters or other environmental samples with this direct conductivity method, high levels of carbonate, interfering metals (e.g. magnesium and calcium) and excess base may cause baseline disturbances. These interferences can be eliminated using an H^+ form SPE system.

Anions by Absorbance Detection

Anions by direct absorbance detection A number of inorganic anions absorb light in the low UV ($205\text{--}215 \text{ nm}$) range. In such cases it is possible to analyse these anions using a standard high performance liquid chromatograph possessing a UV detector. EPA Method B-1011 describes the analysis of nitrite and nitrate in water using absorbance detection at 214 nm . The molar absorptivity of these ions is quite strong (e.g. $9000 \text{ M}^{-1} \text{ cm}^{-1}$ for nitrate at 210 nm), yielding detection limits of 0.003 and 0.0003 mg L^{-1} for nitrite and nitrate respectively. UV detection provides comparable precision and

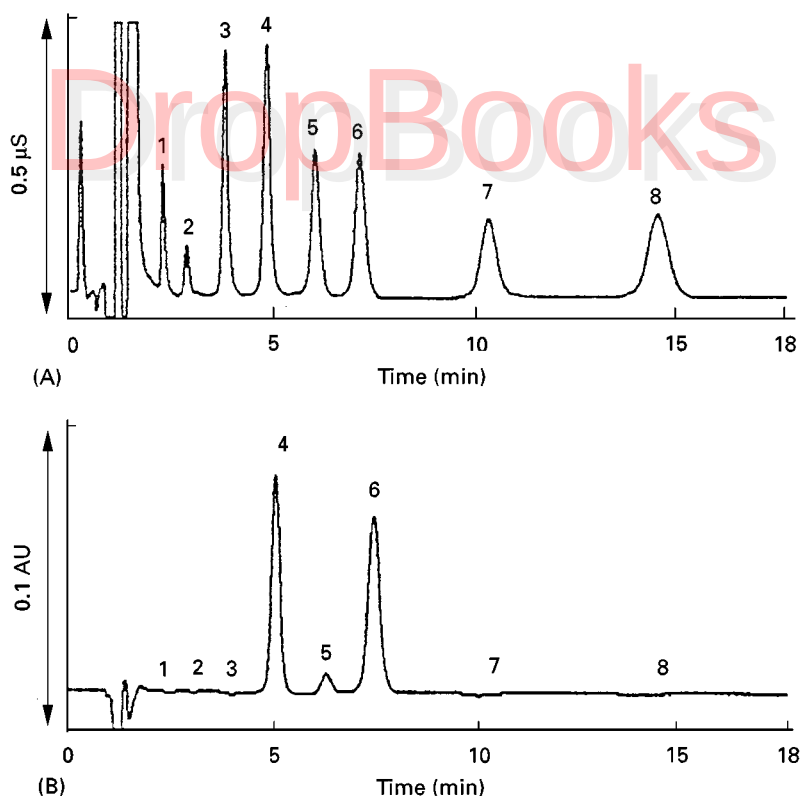


Figure 3 Separation and detection of standard inorganic anions using: (A) direct conductivity detection; (B) direct absorbance detection at 214 nm . Peaks: 1, $1 \mu\text{g L}^{-1}$ fluoride; 2, unknown concentration of bicarbonate (due to contamination by CO_2 in air); 3, $2 \mu\text{g L}^{-1}$ chloride; 4, $4 \mu\text{g L}^{-1}$ nitrite; 5, $4 \mu\text{g L}^{-1}$ bromide; 6, $4 \mu\text{g L}^{-1}$ nitrate; 7, $6 \mu\text{g L}^{-1}$ phosphate; 8, $4 \mu\text{g L}^{-1}$ sulfate. Experimental conditions: column, Waters IC-Pak A HR ($75 \times 4.6 \text{ mm}$; $6 \mu\text{m}$); eluent, borate–gluconate (pH 8.5); flow rate, 1.0 mL min^{-1} ; detection, direct absorbance at 214 nm followed in series by direct conductivity at 35°C ; injection, $100 \mu\text{L}$. (Courtesy of Waters.)

accuracy to suppressed conductivity for nitrate. Other anions such as F^- , Cl^- , ClO_4^- , HPO_4^{2-} and SO_4^{2-} do not absorb significantly above 195 nm, and so do not interfere, as can be seen in Figure 3B.

Other ions which absorb in the UV include Br^- (214 nm; 50 p.p.b. detection limit); BrO_3^- (210 nm; 50 p.p.b.); CrO_4^{2-} ($1600\text{ M}^{-1}\text{ cm}^{-1}$ at 365 nm; < 1 p.p.b.); I^- (226 nm; 50 p.p.b.); and IO_3^- (210 nm; 50 p.p.b.). However, there are no approved methods using UV absorbance detection for these ions.

Anions by indirect absorbance detection Indirect UV absorbance may also be used to detect inorganic anions. In indirect detection, the eluent is a strongly absorbing anion such as phthalate. Within the eluting peak, the analyte anion displaces an equivalent amount of the absorbing eluent anion. Thus, the absorbance decreases as each analyte elutes from the column. Often the leads on the detector are reversed when performing indirect detection, such that the peaks appear positive. Standard anions can be determined in 15 min with low p.p.b. detection limits using 0.8 mmol L^{-1} phthalate at pH 6.8 as eluent and indirect detection at 265 nm.

Post-column reaction detection In EPA Method 218.6 and ASTM Method D5257, hexavalent chro-

mium Cr(VI) is separated from Cr(III) and other reactive metal ions by anion exchange chromatography. Detection is by absorbance at 520 nm after post-column reaction with diphenylcarbohydrazide. This procedure is suitable for monitoring Cr(VI) from 1 to 1000 p.p.b.

Dasgupta has provided an exhaustive review of other methods for post-column reaction detection of anions.

Cations by suppressed conductivity EPA Method 300.7 is currently the only approved procedure for determination of inorganic cations using ion chromatography. This procedure was developed to determine monovalent and divalent cations in precipitation using ion chromatography with suppressed conductivity detection. As indicated in Table 2, detection limits for this procedure are in the low p.p.b. ($\mu\text{g L}^{-1}$) range. Cation exchange with suppressed conductivity is not limited to metal ions. **Figure 4** shows a separation of alkali metals, alkaline earth metals and some volatile amines.

Cations by Direct Conductivity

Alkali metals and alkaline earth metals can be detected using nonsuppressed conductivity detection. Using a

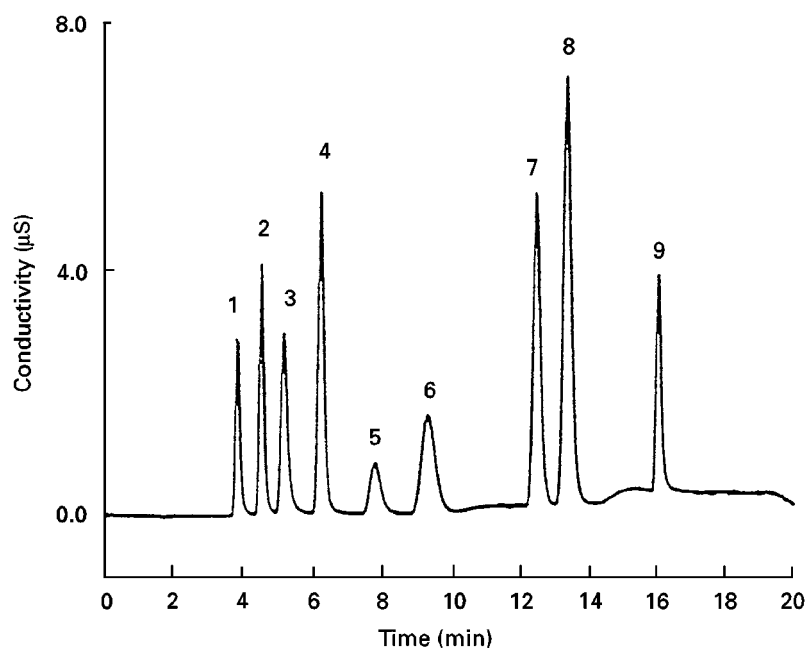


Figure 4 Separation of group I and II cations and volatile amines. Column: 25 cm $\times$ 4 mm, 8–9 μm IonPac CS12A; mobile phase, step gradient of 16 mmol L^{-1} sulfuric acid–4% acetonitrile to 28 mmol L^{-1} sulfuric acid–4% acetonitrile to 50 mmol L^{-1} sulfuric acid–5% acetonitrile; flow rate, 1.0 mL min^{-1} ; detection, conductivity with suppression from a CSRS-II autosuppressor unit; temperature, 40°C . Sample: 1, lithium (0.5 p.p.m.); 2, sodium (2.0 p.p.m.); 3, ammonium (2.5 p.p.m.); 4, potassium (5.0 p.p.m.); 5, morpholine (10.0 p.p.m.); 6, 2-diethylaminoethanol (10.0 p.p.m.); 7, magnesium (2.5 p.p.m.); 8, calcium (5.0 p.p.m.); 9, cyclohexylamine (15.0 p.p.m.). (Courtesy of Dionex.)

3 mmol L⁻¹ HNO₃/0.1 mmol L⁻¹ ethylenediaminetetraacetic acid (EDTA) eluent on a Waters IC-Pak cation M/D column, linear response is observed down to 0.05 p.p.m. for Li⁺, Na⁺, NH₄⁺, K⁺, Mg²⁺ and Ca²⁺ in a 15 min separation. Other alkali metals, alkaline earth metals and amines can also be detected in this fashion.

Inorganic Cations by Absorbance Detection

Cations by direct and indirect absorbance detection

Simple inorganic cations generally cannot be effectively monitored by direct UV absorbance due to their low molar absorptivities. However, metal complexes with complexing ligands such as dithiocarbamates provide high molar absorptivities which yield low p.p.b. detection limits. Furthermore, these hydrophobic complexes can be separated using conventional reversed-phase columns. For instance, pre-derivatization of Ru³⁺, Pt²⁺, Pd²⁺ and Rh³⁺ with diethyldithiocarbamate enables their separation on a standard C₁₈ reversed-phase column with a methanol-water eluent and sensitive (p.p.b.) detection at 260 nm. This approach is most attractive when a post-column reaction detection system cannot be used and for the analysis of precious metals.

Indirect absorbance detection of alkali metals can be performed using eluents such as 4-methylbenzylamine (262 nm) and Cu²⁺ (252 nm). However, the detection limits are inferior to those obtained with suppressed conductivity.

Inorganic cations by post-column reaction detection The most common means of separating the first row transition metals by LC is on reversed-phase

(C₁₈) columns dynamically coated with C₈SO₃⁻ or low capacity ion exchangers. Complexing eluents such as tartrate and pyridine-2,6-dicarboxylate (PDCA) are used. Post-column reaction detection with PAR can be used for a wide range of metal ions including Pb²⁺, Fe³⁺, Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺, Cd²⁺, Mn²⁺, Fe²⁺ and Hg²⁺ with detection limits in the low p.p.b. range for typical injection volumes. This is more than 100 times the sensitivity achievable with conductivity. Figure 5 shows a typical separation of transition metals by HPLC. A number of species (Fe³⁺, Cr³⁺, Sn⁴⁺) elute at the void volume (1.6 min in Figure 5) and so cannot be quantified. Degradation of the peak shape for copper is observed if trace organic acids are present in the water used to prepare the eluent.

High efficiency lanthanide separations are achieved using reversed-phase C₁₈ columns dynamically coated with C₈SO₃⁻ with gradient elution by α -hydroxyisobutyric acid. Elution is in the order of decreasing atomic mass (Lu → La). Post-column reaction detection with Arsenazo III with detection at 658 nm yields 1–5 p.p.b. detection limits using a 20 μ L injection.

Simultaneous separation of transition and lanthanide metals has been achieved based on the charge of the metal-PDCA complexes. Transition metals form monovalent or divalent anionic complexes with PDCA and so can be separated with a PDCA eluent on a Dionex CS5 column. Once all of the transition metals have eluted, the lanthanides, which form strongly retained trivalent anionic complexes with PDCA, are eluted with an oxalate-diglycolate eluent. Detection limits of 20–40 p.p.b. are observed for post-column reaction detection with PAR.

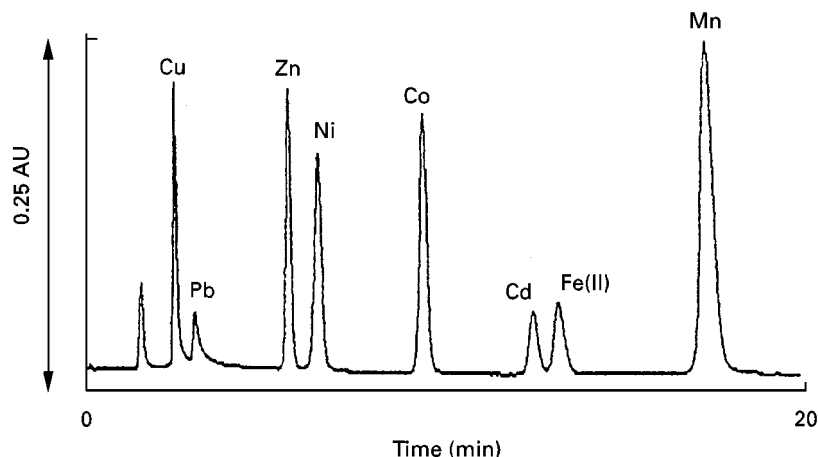


Figure 5 Separation of transition metals with post-column reaction detection. Experimental conditions: column, Waters Delta Pak C₁₈ (10 nm, 5 μ m); eluent, 2 mmol L⁻¹ sodium octanesulfonate, 35 mmol L⁻¹ sodium tartrate, 5% acetonitrile adjusted to pH 3.65 with NaOH; flow rate, 0.8 mL min⁻¹; detection, 500 nm after post-column addition of 0.5 mL min⁻¹ of 0.2 mmol L⁻¹ 4-(2-pyridylazo) resorcinol (PAR), 1 mol L⁻¹ acetic acid, 3 mol L⁻¹ ammonium hydroxide. (Courtesy of Waters.)

See also: II/Chromatography: Liquid: Derivatization; Mechanisms: Ion Chromatography.

Further Reading

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Thin-Layer (Planar) Chromatography

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Introduction

Thin-layer chromatography (TLC) is a subdivision of liquid chromatography in which the mobile phase (a liquid) migrates through the stationary phase (thin layer of porous sorbent on a flat inert surface) by capillary action. In 1938, two Russian workers, Izmailov and Schraiber, separated certain medicinal compounds on binder-free horizontal thin layers of alumina spread on a glass plate. Since the development was carried out by placing solvent drops on the glass plate containing sample and sorbent, their method was called drop chromatography. This method remained unnoticed for 10 years until two American chemists, Meinhard and Hall, used a mixture of aluminium oxide (adsorbent) and celite (binder) in a layer on a microscope slide to separate Fe^{2+} from Zn^{2+} . They called this technique surface chromatography and this was the first application of planar chromatography to the separation of inorganic ions. The real impetus for advancement of TLC started in 1951 with the work of Kirchner and his associates. Stahl introduced the term thin-layer chromatography in 1958 and standardized procedures, materials and nomenclature. The rapid growth of TLC slowed down during the 1970s with the rise in popularity of high performance liquid chromatography (HPLC) and ion chromatography (IC). However, recent improvements in TLC have removed many of its limitations.

High performance TLC (HPTLC) layers, being thinner and made of more uniform particle size sorbents, provide faster separations, reduced zone diffusion, lower detection limits, less solvent consumption and better separation efficiency. The distinct advantages of TLC over HPLC have been identified as low solvent consumption, low operational cost, easier sample preparation, more rapid throughput, greater detection possibilities and the use of disposable plates. TLC permits the simultaneous analysis of many samples in the same time required for one HPLC analysis and samples and standards are analysed by TLC under exactly the same conditions rather than serially, as in HPLC. Typically, 18–36 samples can be run on a single HPTLC plate with a development time of 3–20 min over a migration distance of 2–7 cm. However, the influence of environmental conditions on the reproducibility of R_F values and poor separation efficiency have been major disadvantages of TLC compared with HPLC and gas chromatography (GC).

TLC can be used for *qualitative* analysis, to identify the presence or absence of a particular substance in a mixture; *quantitative* analysis, to determine precisely and accurately the amount of a particular substance in a sample mixture; and *preparative* analysis, to purify and isolate a particular substance for subsequent use. All three applications require the common procedures of sample application, chromatographic separation and component visualization. However, analytical TLC differs from preparative TLC in that volumes and/or weights of samples are applied to thicker layers in the latter case.